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**Print: Pore-forming molecules fight cystic fibrosis**

**Online: Pore-forming small molecules might offer a way to tackle cystic fibrosis**

In cystic fibrosis, ion-transport abnormalities cause problems in many organs. A small molecule that forms cell-membrane pores which allow ion transport, shows promise in studies of human cells and an animal model of the disease. See Letter p.405

David N. Sheppard & Anthony P. Davis,

In cystic fibrosis, abnormalities in the ion-channel protein CFTR causes problems in transport of the ions chloride ( $\text{Cl}^-$ ) and bicarbonate ( $\text{HCO}_3^-$ ) in lung epithelial cells, which results in a thick build-up of airway mucus. This hinders the normal process that removes mucus, and the inhaled bacteria trapped within it, from the airways, and this blockage leads to persistent infection and inflammation, which destroys lung tissue<sup>1</sup>. Writing in *Nature*, Muraglia *et al.*<sup>2</sup> demonstrate that a small molecule called amphotericin B, which can form an ion channel in the cellular membrane of lung cells, effectively restores ion transport and antibacterial defences when tested using human cells from people who have cystic fibrosis and an animal model of the disease.

Much progress is currently being made in efforts to develop new clinical treatments for cystic fibrosis. Cocktails of three drugs are now in clinical trials that might potentially greatly slow the progression of the disease<sup>3,4</sup>. Problems in CFTR reaching its usual location on the cell membrane is a common defect in cystic fibrosis, and two of the drugs help CFTR

to reach the cell membrane while the third one boosts ion transport through the channel. However, approximately 1,800 faulty versions of the CFTR-encoding gene associated with disease have been identified so far, and this diversity of mutations might mean that CFTR-targeting drugs will not work for everyone who has the disease<sup>1</sup>.

Therefore interest has grown in trying to find widely applicable treatment options for cystic fibrosis. For example, efforts are being made to try to find ways to restore ion transport that bypass faulty or missing CFTR proteins by using other pathways to transport negatively charged ions (anions) out of the cell. For example, using anion channels that are found naturally in lung epithelial cells<sup>5</sup> or exploiting synthetic molecules that bind anions selectively and function either as artificial anion channels<sup>6</sup> or transporters that shuttle anions across a lipid membrane<sup>7</sup>.

$\text{HCO}_3^-$  has crucial roles in lung-defence mechanisms and abnormalities in the secretion of  $\text{HCO}_3^-$  from lung cells into the airways underlie many of the symptoms of cystic fibrosis. Studies of a pig model of cystic fibrosis have indicated that the absence of normal  $\text{HCO}_3^-$  transport into the airways from lung cells prevents bacterial killing by antimicrobial factors and affects the pH and viscosity of the airway surface liquid that covers lung cells<sup>8</sup>. This liquid surrounds cellular protrusions called cilia (Fig. 1a) that beat to transport away the overlying mucus<sup>8</sup>.  $\text{HCO}_3^-$  is essential too for the untangling of mucins during mucus formation<sup>9</sup>. In cystic fibrosis, the volume of airway surface liquid is lower than normal and is more acidic than normal (Fig. 1b) due to the defects in anion secretion<sup>1,8</sup>.

Cellular transport proteins cause the anions  $\text{Cl}^-$  and  $\text{HCO}_3^-$  to accumulate in lung epithelial cells, and in people who have cystic fibrosis, these anions remain trapped within the

cells. As a result, the high concentration of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  inside cells and their low concentration in the airway surface liquid generates a steep concentration gradient for these anions across the upper (apical) surface of lung cells that might suffice to drive anion exit without requiring an energy source for transport. Muraglia *et al.*<sup>2</sup> therefore reasoned that a small-molecule  $\text{HCO}_3^-$  transporter could exploit this concentration gradient to restore  $\text{HCO}_3^-$  transport and thereby also restore the defence processes that depend on  $\text{HCO}_3^-$  in the lungs of people who have cystic fibrosis. But which small molecule to use?

Muraglia and colleagues focused on an anti-fungal agent called amphotericin B that is made naturally by bacteria. This small molecule might seem a peculiar choice at first sight. It forms non-selective ion channels that are permeable to both anions and positive ions (cations) and it can be toxic to human cells<sup>10</sup>. However, three lines of evidence made a persuasive case for this choice. First, its ion-channel function can be separated from its anti-fungal activity by judicious control of the amphotericin B concentration<sup>10</sup>; which suggests that the toxicity can be managed. Second, amphotericin B restored the transport of potassium ions in yeast cells lacking a potassium transporter protein, demonstrating that it can provide a functional replacement for natural transport proteins<sup>11</sup>. Third, Muraglia *et al.* demonstrated that amphotericin B transported  $\text{HCO}_3^-$  across artificial lipid membranes.

Muraglia and colleagues added amphotericin B to the apical membrane of human lung cells that are a standard *in vitro* model system for studying cystic fibrosis, and this treatment resulted in  $\text{HCO}_3^-$  secretion from the cells, caused the pH of the airway surface liquid to rise and restored the volume of airway surface liquid to a normal value (Fig. 1c), compared to the effect in cells that did not receive amphotericin B. The authors also tested lung epithelial cells grown *in vitro* from tissue donated by people who represented a range of CFTR variants. The

addition of amphotericin B to the apical surface of these cells resulted in an increase in the pH, a decrease in the viscosity of the airway surface liquid and an enhancement of bacterial killing power compared to the effect in the cells that did not receive amphotericin B. Finally, Muraglia *et al.* demonstrated in an *in vivo* pig model of cystic fibrosis, that if animals received the drug AmBisome, which contains amphotericin B and is already in use in the clinic, this increased the pH of the airway surface liquid, compared to the pH of this liquid in animals who did not receive the drug treatment.

This study by Muraglia *et al.* and other work<sup>12</sup> offer a proof-of-concept that small molecules can function as surrogates for defective or deficient transport proteins in human disease. Although a small molecule cannot replicate all the functions of a complex protein, the success of this approach will surely encourage wider exploration of such uses of small molecules. Why did amphotericin B work so well? The authors report that if a protein called the Na<sup>+</sup>, K<sup>+</sup>-ATPase, an ion transport pump located on the tissue-facing (basolateral) membrane of lung epithelial cells was inhibited in human lung epithelial cells grown *in vitro* from people who have cystic fibrosis, this prevented the beneficial effects of amphotericin B treatment. Na<sup>+</sup>, K<sup>+</sup>-ATPase activity affects cellular anion transport and the movement of ions it regulates affects ion transport through other transport proteins, including the import of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>. The data suggesting that the action of amphotericin B in the apical membrane required the activity of ion-transport proteins located in the basolateral membrane, is an example of what is called transcellular cross-talk between epithelial membranes<sup>13</sup>, in which ion entry and exit through the different surfaces of the cell is regulated to prevent cellular damage.

Muraglia and colleagues' work raise many intriguing questions that will no doubt stimulate future research. For example, how much amphotericin B would be needed to fully restore host defences? Could it be used in combination with drugs that rescue faulty CFTR proteins? And would it be safe to use amphotericin B routinely throughout an individual's life? As new therapeutic approaches are developed for people who have cystic fibrosis, this might lead to improvements not only for the treatment of all people with this disease but perhaps also for other lung conditions, which have disease characteristics that are similar to those of cystic fibrosis.

**David N. Sheppard** is in the School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol BS8 1TD, UK. **Anthony P. Davis** is in the School of Chemistry, University of Bristol, Bristol BS8 4LN, UK.  
e-mails: [D.N.Sheppard@bristol.ac.uk](mailto:D.N.Sheppard@bristol.ac.uk); [Anthony.Davis@bristol.ac.uk](mailto:Anthony.Davis@bristol.ac.uk)

**D.N.S. declares competing financial interests. See [go.nature.com/xxx](https://go.nature.com/xxx) for details.**

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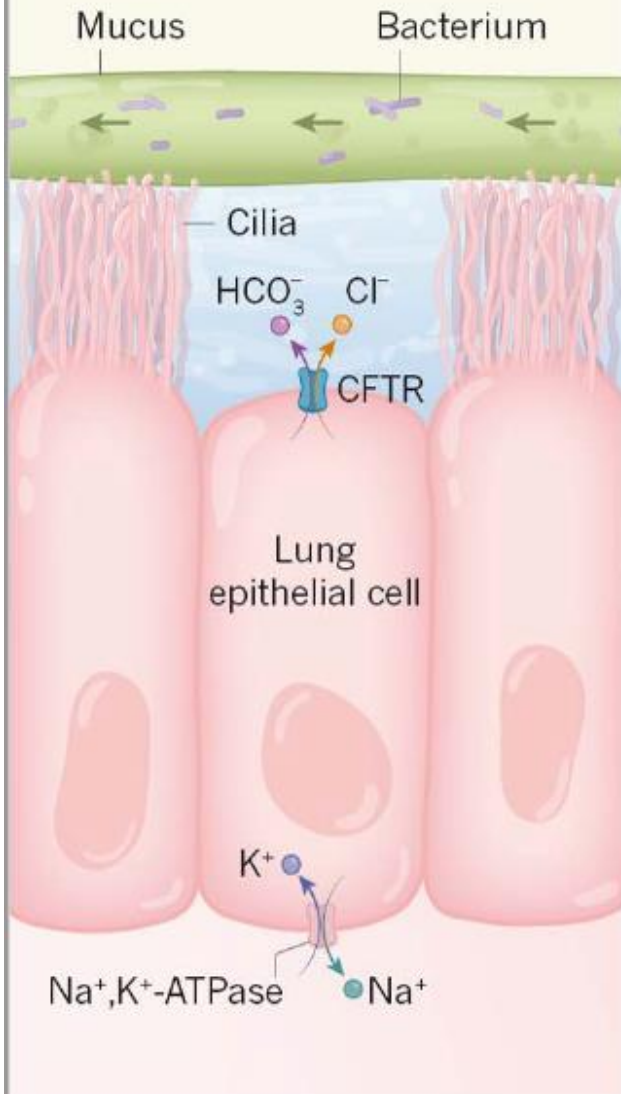
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**Figure 1 | The small molecule amphotericin B can tackle lung problems that occur in cystic fibrosis.** **a**, Lung epithelial cells are bathed in a layer of airway surface liquid on which mucus floats. Cellular protrusions called cilia beat to propel (arrows) this mucus and trapped bacteria from the airways. Mucus removal, the killing of trapped bacteria and the maintenance of a normal volume of airway surface fluid at physiological pH requires the presence of

$\text{HCO}_3^-$  and  $\text{Cl}^-$  due to activity of a network of cellular proteins that transport ions. In certain lung epithelial cells, these include the protein CFTR in the fluid-facing (apical) membrane, which transports  $\text{HCO}_3^-$  and  $\text{Cl}^-$  out of the cell, and the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase protein in the tissue-facing (basolateral) membrane of the cell. **b**, In cystic fibrosis, CFTR is absent from the apical membrane of epithelial cells, which causes a build-up of  $\text{HCO}_3^-$  and  $\text{Cl}^-$  within these cells (due to the action of a network of ion transport proteins (not shown) that also depend on the presence of functional  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase). The absence of secretion of  $\text{HCO}_3^-$  and  $\text{Cl}^-$  results in the formation of airway surface fluid that is more acidic than normal (red), of a smaller volume than normal, and thicker mucus than usual and abnormalities in mucus removal and in the killing of bacterial cells within the mucus. **c**, Muraglia *et al.*<sup>2</sup> demonstrate in studies of human cells and in a pig model of cystic fibrosis that if the small molecule ion channel amphotericin B (AmB) is used to treat cells that lack CFTR on their apical surface, amphotericin B restores the missing ion transport and can prevent the problems that arise when CFTR is absent from the apical surface of lung cells.



**a Normal lung**



**b Cystic fibrosis**



**c Cystic fibrosis + AmB treatment**

